solvent was removed under a stream of nitrogen, and the residue was dissolved in 1 mL of CH_2Cl_2 and injected onto a preparative HPLC column, as for FENP, to yield 2.5 mCi (4.3%, decay corrected) of [^{18}F]FENP (t_R 25 min). See Figure 1 for a radio- and UV chromatogram for a similar, though smaller scale, reaction.

Acknowledgment. We are grateful for support of this work through grants from the Department of Energy (Grant DE-FG02-86ER60401 to J.A.K. and Grant DE-FG02-84ER60218 to M.J.W.) and the National Institutes of Health (Grant HL13851). We are thankful to Dr. F. Zeelen, of Organon, for providing us with samples of ORG 2058 and ORG OH-06 (FENP) and to K. E. Carlson for assistance.

Registry No. 1, 113975-10-3; ORG 2058, 24320-06-7; FENP, 25908-76-3; [^{18}F]FENP, 113948-68-8.

Cardiotonic Agents. Synthesis and Inotropic Activity of a Series of Isoquinolin-3-ol Derivatives

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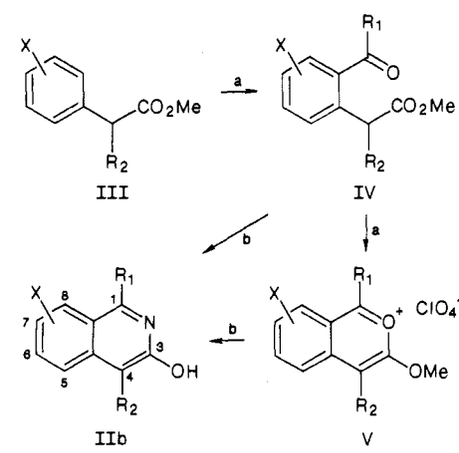
A series of isoquinolin-3-ol derivatives (II) was prepared as analogues of the clinical cardiotonic agent bemarinone (ORF 16600, I). Although in many respects the structural requirements for the cardiotonic activity of II are similar to those of bemarinone, certain differences between the series were noted. Our structure–activity studies show that II is less sensitive to alkoxy-substitution effects than is I, and more significantly, 4-substitution of II by alkyl groups, halogen, or alkanecarboxylic acid derivatives enhances cardiotonic activity in II in contrast to I, wherein analogous substitution eliminated activity. A linear correlation between contractile force (CF) increase and cyclic nucleotide phosphodiesterase fraction III (PDE-III) inhibition by the title compounds was determined. The isoquinoline derivatives were characteristically short-acting cardiotonic agents with good potency and selectivity.

Our laboratory has been investigating novel quinazolinones for a number of years,[1] and recently we reported the synthesis[2] and cardiotonic activity[3,4] of bemarinone (ORF 16600, I). Compound I was the most potent cardiotonic agent of the series in which structure–activity relationship (SAR) studies revealed that (1) 5,6-dimethoxy and 5,6-methylenedioxy substitution produced the best activity, (2) hydrogen at N-1 (rather than alkyl) was essential, (3) there was moderate bulk tolerance at C-4 with methyl, ethyl, and isopropyl substitution, all producing good activity, and (4) a good correlation was found between cardiotonic activity and phosphodiesterase fraction III (PDE-III) inhibition although PDE-III inhibition alone was not predictive of cardiotonic activity for this series.

We became interested in evaluating related compounds that were isosteric with I. In particular, isoquinoline analogues such as II in which the N-1 position is replaced by a carbon were especially interesting since methods of synthesis for such systems are known[5] and several alkaloid isoquinoline derivatives such as papaverine, dioxyline, and ethaverine,[6] as well as some related derivatives,[7] are known to possess interesting cardiovascular pharmacology. Compounds of structure II may exist as a lactam (IIa) or lactim (IIb) tautomer among other possibilities,[8,9] either or all of which might have interesting pharmacological properties. The lactim form appears to be preferred in nonhydroxylic solvents,[9] although this tautomerism also depends upon substitution on the heterocyclic ring. Of particular interest for our study are the various alkoxy-

Scheme I[a]



[a] (a) $(R_1CO)_2O/HClO_4/0$ °C to room temperature, (b) NH_4OH or NH_4OAc.

(1) Bandurco, V. T.; Malloy-Wong, E.; Levine, S. D.; Hajos, Z. G. *J. Med. Chem.* **1981**, *24*, 1455.
(2) Press, J. B.; Bandurco, V. T.; Wong, E. M.; Hajos, Z. G.; Kanojia, R. M.; Mallory, R. A.; Deegan, E. G.; McNally, J. J.; Roberts, J. R.; Cotter, M. L.; Garden, D. W.; Lloyd, J. R. *J. Heterocycl. Chem.* **1986**, *23*, 1821.
(3) Bandurco, V. T.; Schwender, C. F.; Bell, S. C.; Combs, D. W.; Kanojia, R. M.; Levine, S. D.; Mulvey, D. M.; Appollina, M. A.; Reed, M. S.; Malloy, E. A.; Falotico, R.; Moore, J. B.; Tobia, A. J. *J. Med. Chem.* **1987**, *30*, 1421.
(4) (a) Falotico, R.; Moore, J. B.; Tolman, E. L.; Bandurco, V. T.; Bell, S. C.; Tobia, A. J. *Pharmacologist* **1985**, *27*, abstr. 679. (b) Falotico, R.; Moore, J. B.; Bandurco, V. T.; Bell, S. C.; Levine, S. D.; Tobia, A. J. *Drug Dev. Res.*, in press.
(5) For a general review of approaches to the synthesis of isoquinolines, see, for examples: Kametani, T. In *The Total Synthesis of Natural Products, Volume 3*; ApSimon, J., Ed.; Wiley: New York, 1977; Chapter 1.
(6) Hanna, C.; Parker, R. C. *Arch. Int. Pharmacodyn. Ther.* **1960**, *126*, 386.
(7) Kreighbaum, W. E.; Kavanaugh, W. F.; Comer, W. T.; Deitchman, D. *J. Med. Chem.* **1972**, *15*, 1131.
(8) McCorkindale, N. J.; McCulloch, A. W. *Tetrahedron* **1971**, *27*, 4653.
(9) Evans, D. A.; Smith, G. F.; Wahid, M. A. *J. Chem. Soc. B* **1967**, 590.

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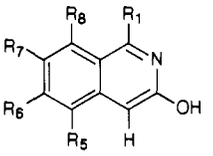
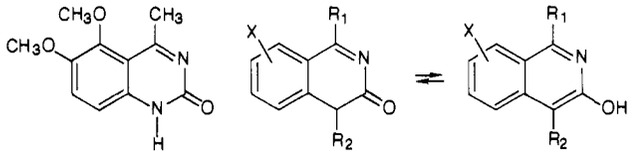
Table I. Isoquinolin-3-ols without 4-Substitution



compd	R_1	R_5	R_6	R_7	R_8	empirical formula[a]	mp,[b] °C	dose[c]	CF[d]	dP/dt[e]	HR[f]	MAP[g]
1	Me	H	OMe	OMe	H	$C_{12}H_{13}NO_3$	280–282[h]	1.75	100	55	25	−32
2	Me	H	H	OMe	OMe	$C_{12}H_{13}NO_3$	165–166	0.875	62	51	10	−6
3	Me	H	O-CH_2-O		H	$C_{11}H_9NO_3$	290–293[i]	3.75	82	32	13	−10
4	Me	H	OMe	OMe	OMe	$C_{13}H_{15}NO_4$	232–233	0.875	76	45	13	−2
5	Me	H	OMe	H	OMe	$C_{12}H_{13}NO_3$	280–282 dec	0.875	91	62	13	−18
6	Me	H	OMe	H	H	$C_{11}H_{11}NO_2$	255–258 dec	0.925	65	56	8	0
7	Me	H	OMe	OEt	H	$C_{13}H_{15}NO_3 \cdot {}^1/_4H_2O$	235–240 dec	0.875	197	88	22	−10
8	Me	H	OMe	OBu	H	$C_{15}H_{19}NO_3$	213–214	0.875	182	58	26	−18
9	Me	OMe	OMe	H	H	$C_{12}H_{13}NO_3$	216–218 dec	1.875	28	13	3	1
10	Me	H	OEt	OEt	H	$C_{14}H_{17}NO_3$	231–232 dec	0.875	84	42	15	−11
11	Me	H	H	OMe	H	$C_{11}H_{11}NO_2 \cdot {}^1/_4H_2O$	227–230	0.875	114	87	19	−18
12	H	H	H	OMe	OMe	$C_{11}H_{11}NO_3 \cdot {}^1/_8H_2O$	130–132	8.75	60	34	13	−7
13	H	H	OMe	OEt	H	$C_{12}H_{13}NO_3$	248–250	1.875	34	32	7	0
milrinone								0.100	105 ± 9	76 ± 11	15 ± 2	−13 ± 3
bemarinone								0.375	71 ± 7	30 ± 10	11 ± 2	−6 ± 2

[a] All compounds exhibited satisfactory (±0.4%) elemental analysis except where noted. [b] General procedure in A in the Experimental Section. [c] Cumulative dose in mg/kg iv given to anesthetized open-chest dogs. [d] Contractile force as percent increase from pretreatment control values; standard error of ±10% for CF values. [e] dP/dt as percent increase from pretreatment control values; standard error of ±20% for dP/dt values. [f] Heart rate as percent change from pretreatment control values; standard error of ±20% for HR values. [g] Mean arterial blood pressure as percent change from pretreatment controls; standard error of ±10% for MAP values. [h] Carbon: calcd 65.74, found 65.26. [i] Carbon: calcd 65.02, found 64.53.

substituted derivatives II (X = RO, $(RO)_2$, etc.) since these compounds relate most directly both to bemarinone and to the naturally occurring biologically active alkaloids.



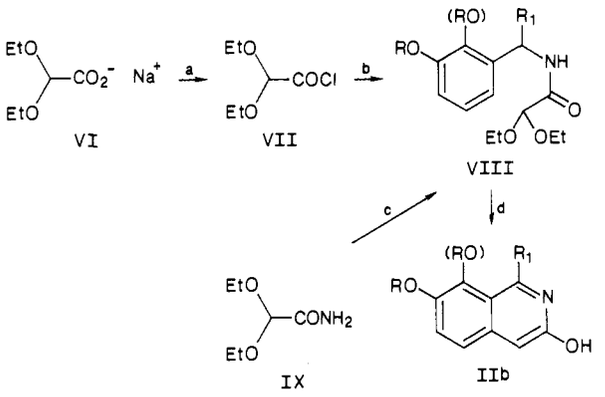
I IIa IIb

Chemistry

The first synthesis of IIb (R_2 = H, X = 6,7-dimethoxy) was reported by Bentley et al.[10] and is essentially that shown in Scheme I. In their report, keto ester IV was prepared by Friedel–Crafts reaction with acetyl chloride in carbon disulfide and was subsequently hydrolyzed to the corresponding acid. The acid was then treated with aqueous ammonium hydroxide to give the product in an unreported yield. The 3-hydroxy tautomeric structure was inferred from the formation of an *O*-acetate as well as from a positive ferric chloride reaction and solubility in dilute sodium hydroxide.

For our work in the preparatin of 6-alkoxy and 6,7-dialkoxy derivatives, we found it convenient to react appropriately substituted arylacetic acid esters III or the corresponding nitrile in neat acid anhydride (or diluted with methylene chloride to control the exotherm) with a Lewis acid catalyst such as boron trifluoride or preferably perchloric acid to produce benzopyrylium salt V, which formed in nearly quantitative yield presumably via IV.[11] The salts V were isolable and stable for several weeks. Reaction of V with concentrated ammonium hydroxide or with ammonium acetate led to rapid formation of IIb in 60–70% yield.

Scheme II[a]



VI VII VIII IX IIb

[a] (a) $SOCl_2$, (b) substituted benzylamine/pyridine/reflux, 1 h, (c) NaH, NaI, THF/substituted benzyl chloride, reflux overnight, (d) PPA/100 °C, 1.5 h.

The 7-alkoxy and 7,8-dialkoxy derivatives were prepared by a Pomeranz–Fritsch type cyclization[12a] as shown in Scheme II. In the first approach to this synthesis,[12b] sodium diethoxyacetate (VI, prepared from commercially available acid) was treated with thionyl chloride and the resultant acid chloride VII was reacted with an appropriately substituted benzylamine to give amide VIII. Alternatively, amide VIII could be prepared by reacting the anion of diethoxyacetamide with an appropriately substituted benzyl chloride. Ring closure of VIII to IIb was effected in modest yield by the action of polyphosphoric acid.

Biology

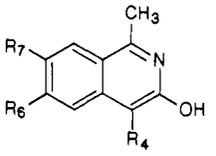
Cardiotonic activity of the title compounds was evaluated in anesthetized open chest dogs by using procedures previously described.[3,13] The effect of intravenous (iv)

(10) Bentley, H. R.; Dawson, W.; Spring, F. S. *J. Chem. Soc.* **1952**, 1763.

(11) Dorofeenko, G. N.; Korobkova, V. G.; Krivum, S. V. *Khim. Geterotsikl. Soedin, Sb. 2* **1970**, 200; *Chem. Abstr.* **1972**, *76*, 140460*u*.

(12) (a) For a review, see: Bobbitt, J. M. *Adv. Heterocycl. Chem.* **1973**, *15*, 99. (b) Fukumi, H.; Kurihara, H. *Heterocycles* **1978**, *9*, 1197.

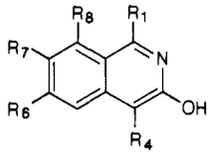
Table II. 4-Alkyl- and 4-Haloisoquinolinols



compd	R_4	R_6	R_7	empirical formula[a]	mp,[b] °C	dose[c]	CF[d]	dP/dt[e]	HR[f]	MAP[g]
14	Pr	OMe	OMe	$C_{15}H_{19}NO_3$	253–255	0.875 iv	108	55	22	4
14						1.00 id	20	20	5	3
15	Et	OMe	OMe	$C_{14}H_{17}NO_3$	272–274	0.025 iv	143	98	13	−12
15						1.00 id	132	92	22	−22
16	Me	OMe	OMe	$C_{13}H_{15}NO_3$	295–297 dec	0.10 iv	80	48	15	−4
16						1.00 id	64	30	8	−6
17	Br	OMe	OMe	$C_{12}H_{12}BrNO_3 \cdot HBr \cdot H_2O$	175–178[h,i]	0.875 iv	128	25	26	−40
18	Cl	OMe	OMe	$C_{12}H_{12}ClNO_3$	>300[i]	0.875 iv	89	88	29	1
19	*i*-Pr	OMe	OMe	$C_{15}H_{19}NO_3$	223–225	0.050 iv	76	54	6	−5
19						1.00 id	70	100	21	−16
20	Cl	O–CH_2–O		$C_{11}H_8ClNO_3$	>300[i]	1.875 iv	88	51	28	−22
21	*i*-Bu	OMe	OMe	$C_{16}H_{21}NO_3$	213–214	1.875 iv	39	25	10	−6
22	c-C_5H_9	OMe	OMe	$C_{17}H_{21}NO_3$	282–284 dec	1.875 iv	46	22	5	3
23	Et	OMe	OEt	$C_{15}H_{19}NO_3$	250–252	0.875 iv	118	6	26	−46
milrinone						0.100 iv	105 ± 9	76 ± 11	15 ± 2	−13 ± 3
milrinone						0.100 id	85 ± 13	36 ± 13	14 ± 6	−3 ± 2
bermarinone						0.375 iv	71 ± 7	30 ± 10	11 ± 2	−6 ± 2

[a] All compounds exhibited satisfactory (±0.4%) elemental analysis except where noted. [b] General procedure A in the Experimental Section except where noted. [c] Cumulative dose in mg/kg iv or id given to anesthetized open-chest dogs. [d] Contractile force as percent increase from pretreatment control values; standard error of ±10% for CF values. [e] dP/dt as percent increase from pretreatment control values; standard error of ±20% for dP/dt values. [f] Heart rate percent change from pretreatment control values; standard error of ±20% for HR values. [g] Mean arterial blood pressure as percent change from pretreatment controls; standard error of ±10% for MAP values. [h] Carbon: calcd 36.29; found 35.77. Bromine: calcd, 40.28; found, 39.45. [i] General procedure B in the Experimental Section.

Table III. 4-(Carboxyalkyl)-substituted Isoquinolin-3-ols



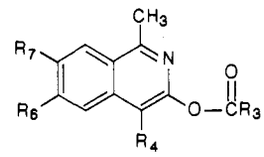
compd	R_1	R_4	R_6	R_7	R_8	empirical formula[a]	mp,[b] °C	dose[c]	CF[d]	dP/dt[e]	HR[f]	MAP[g]
24	Me	$(CH_2)_2CO_2Me$	OMe	OMe	H	$C_{16}H_{19}NO_5$	198–200	0.875 iv	140	78	34	−12
24								10.0 id	105	52	12	−11
25	Me	$(CH_2)_2CO_2H$	OMe	OMe	H	$C_{15}H_{17}NO_5 \cdot HCl$	251–253	1.875 iv	75	42	5	−3
26	Me	$(CH_2)_2CO_2Me$	OMe	OMe	OMe	$C_{17}H_{21}NO_6$	170–171	0.875 iv	22	20	8	−4
27	Et	$(CH_2)_2CO_2Me$	OMe	OMe	H	$C_{17}H_{21}NO_5$	181–183	0.875 iv	47	33	10	−3
28	Me	$(CH_2)_2CO_2Me$	O–CH_2–O		H	$C_{15}H_{15}NO_5$	213–216	0.875 iv	131	118	38	−21
29	Pr	$(CH_2)_2CO_2Me$	OMe	OMe	H	$C_{18}H_{23}NO_5$	194–196	1.875 iv	17	19	2	−4
30	*i*-Pr	$(CH_2)_2CO_2Me$	OMe	OMe	H	$C_{18}H_{23}NO_5$	159–160	1.875 iv	15	17	7	3
31	H	$(CH_2)_2CO_2Me$	OMe	OMe	H	$C_{15}H_{17}NO_5$	178–180	0.875 iv	53	24	7	−4
32	Me	$(CH_2)_2CO_2$-*i*-Pr	OMe	OMe	H	$C_{18}H_{23}NO_5$	188–189	1.875 iv	22	15	3	−6
33	Me	$(CH_2)_2CO_2Et$	OMe	OMe	H	$C_{17}H_{21}NO_5$	178–180	0.875 iv	60	20	8	−4
34	H	$(CH_2)_2CO_2Me$	O–CH_2–O		H	$C_{14}H_{13}NO_5$	208–212	1.875 iv	26	28	6	0
35	Me	$(CH_2)_2CONH_2$	OMe	OMe	H	$C_{15}H_{18}N_2O_4$	259–261	1.875 iv	49	29	11	−9
36	Me	CH_2CO_2Me	OMe	OMe	H	$C_{15}H_{17}NO_5$	243–245	0.875 iv	42	28	6	−1
37	Me	$(CH_2)_2CONHMe$	OMe	OMe	H	$C_{16}H_{20}N_2O_4 \cdot {}^1/_4H_2O$	265–267	1.875 iv	62	40	7	−5
38	Me	$(CH_2)_2CONMe_2$	OMe	OMe	H	$C_{17}H_{22}N_2O_4$	214–216	1.875 iv	16	−5	−2	−14
39	Me	CO_2Me	OMe	OMe	H	$C_{14}H_{15}NO_5$	249–251 dec	1.875 iv	39	19	6	−3
40	Me	CONHMe	OMe	OMe	H	$C_{14}H_{16}N_2O_4 \cdot {}^1/_4H_2O$	316–318 dec	1.875 iv	2	17	0	4
milrinone								0.100 iv	105 ± 9	76 ± 11	15 ± 2	−13 ± 3
milrinone								0.100 id	85 ± 13	36 ± 13	14 ± 6	−3 ± 2
bemarinone								0.375 iv	71 ± 7	30 ± 10	11 ± 2	−6 ± 2

[a] All compounds exhibited satisfactory (±0.4%) elemental analysis except where noted. [b] General procedure A in the Experimental Section. [c] Cumulative dose in mg/kg iv or id given to anesthetized open-chest dogs. [d] Contractile force as percent increase from pretreatment control values; standard error of ±10% for CF values. [e] dP/dt as percent increase from pretreatment control values; standard error of ±20% for dP/dt values. [f] Heart rate as percent change from pretreatment control values; standard error of ±20% for HR values. [g] Mean arterial blood pressure as percent change from pretreatment controls; standard error of ±10% for MAP values.

infusion or intraduodenal (id) bolus administration of compounds on contractile force (CF), dP/dt, heart rate (HR), and mean arterial blood pressure (MAP) was measured. The effects were compared to pretreatment control values and are reported as percent change for the rated activity. All of the compounds tested had a rapid onset of action which was within 1 min of drug infusion. The data reported in Tables I–V are the peak increases that were observed for the administered dose. In almost all cases, the peak effect occurred immediately at the end of the drug infusion. Some of the compounds in this study

(13) Alousi, A. A.; Farah, A. I.; Lesher, G. Y.; Opalka, C. J. *Circ. Res.* **1979**, *45*, 666.

Table IV. Acyl Derivatives of Isoquinolin-3-ols



compd	R_3	R_4	R_6	R_7	empirical formula[a]	mp,[b] °C	dose[c]	CF[d]	dP/dt[e]	HR[f]	MAP[g]
41	OMe	H	OMe	OMe	$C_{14}H_{15}NO_5$	147–149	0.375	119	66	19	−8
42	Me	Br	OMe	OMe	$C_{14}H_{14}BrNO_4$	194–196	0.375	117	60	15	−33
milrinone							0.100	105 ± 9	76 ± 11	15 ± 2	−13 ± 3
bemarinone							0.375	71 ± 7	30 ± 10	11 ± 2	−6 ± 2

[a] All compounds exhibited satisfactory (±0.4%) elemental analysis except where noted. [b] Prepared according to procedure C in the Experimental Section. [c] Cumulative dose in mg/kg iv given to anesthetized open-chest dogs. [d] Contractile force as a percent increase from pretreatment control values; standard error of ±10% for CF values. [e] dP/dt as percent increase from pretreatment control values; standard error of ±20% for dP/dt values. [f] Heart rate as percent change from pretreatment control values; standard error of ±20% for HR values. [g] Mean arterial blood pressure as percent change from pretreatment controls; standard error of ±10% for MAP values.

Table V. Phosphodiesterase Fraction III IC_{50} Values and Contractile Force ED_{50} Values for Selected Isoquinolin-3-ols

compd	ED_{50} CF[a]	PDE-III IC_{50}[b]
5	375	80
6	600	160
8	130	37
14	125	5
15	12	2
16	40	10
17	185	10
18	120	62
19	25	7
22	1875	128
23	20	2
24	165	35

[a] Dose in μg/kg, iv to induce a 50% increase over control level of contractile force in one to three anesthetized dogs determined graphically or by linear regression analysis of the dose–response relationship. [b] Concentration in μM to effect 50% inhibition of cyclic nucleotide phosphodiesterase fraction III activity. Experiments were performed on a single enzyme preparation, in duplicate, with multiple trials using several standards as internal controls. The IC_{50} values are the result of at least two separate determinations at several inhibitor concentrations and are reported with a precision of ±15% (SEM) or better.

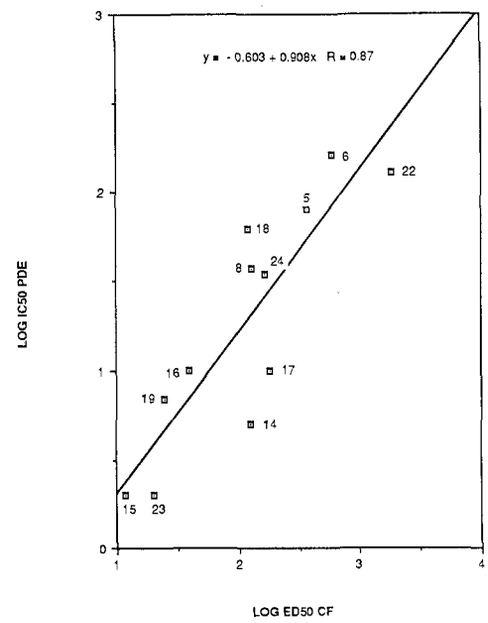


Figure 1. Log–log plot of PDE fraction III IC_{50} vs contractile force ED_{50} (data listed in Table V).

were also evaluated for their inhibition of canine cyclic nucleotide phosphodiesterase fraction III (PDE-III).[14] Data for this assay are reported as an IC_{50} value in μM, the concentration of test compound required to inhibit 50% of the phosphodiesterase activity. Data for these assays are summarized in Tables I–V.

The first compounds prepared and studied were those closest in structure to bemarinone (I) and are summarized in Table I. Analogue 2, most closely resembling bemarinone, had interesting cardiotonic activity but was somewhat less potent than bemarinone.[3] The overall profile of the compounds was similar in that both significantly increased CF and dP/dt while only slightly increasing HR with some vasodilation as evidenced by a small reduction of MAP. Interestingly, while the activity of I was closely correlated with the position of the dimethoxy-substitution pattern, the isoquinolin-3-ol systems were generally less sensitive to alkoxy-substitution effects. Thus 6,7-dimethoxy (**1**), 6,8-dimethoxy (**5**), 6,7,8-trimethoxy (**4**), 7-methoxy (**11**) as well as 6-methoxy (**6**) derivatives all had similar activities at similar doses. Aromatic substitution does indeed influence cardiotonic activity as seen by the fact that the 6,7-methylenedioxy (**3**) and 5,6-dimethoxy (**9**) compounds were less potent. Furthermore, although replacement of 6,7-dimethoxy substitution (**1**) by diethoxy (**10**) had little effect on potency, there was a dramatic increase in activity when only the substituent at C-7 was altered. Thus 6-methoxy-7-ethoxy (**7**) and 6-methoxy-7-butoxy (**8**) substitution substantially increased the cardiotonic potency of the system.

Replacement of the C-1 methyl group by hydrogen was examined in two of the alkoxy series (**12**, **13**) and was found to dramatically reduce cardiotonic activity in each case. In further contrast to the SAR previously noted for the quinazolinones related to I,[3] the isoquinolin-3-ols did not require a hydrogen at the 4-position for activity and had enhanced cardiotonic activity when substituted at C-4 (Table II). Alkyl groups such as methyl (**16**), ethyl (**15**), propyl (**14**), and isopropyl (**19**) at C-4 greatly increased cardiotonic activity relative to **1** while bulkier groups such as isobutyl (**21**) and cyclopentyl (**22**) reduced potency. Halogens such as chloro (**18**, **20**) and bromo (**17**) had less effect on cardiotonic activity but seemed to increase the generalized vasodilatory properties of the system since all three raised HR and two lowered MAP. While replacement of 7-methoxy by 7-ethoxy enhanced cardiotonic activity in the C-4 unsubstituted system without significantly increasing vasodilation, the analogous 4-ethyl-substituted

(14) Thompson, W. J.; Terasaki, W. L.; Epstein, P. M.; Strada, S. J. *Adv. Cyclic Nucleotide Res.* **1979**, *10*, 69.

6-methoxy-7-ethoxy derivative **23** was actually less potent than the 6,7-dimethoxy derivative **15** as a cardiotonic while having enhanced vasodilatory properties. In the 4-alkyl series, **15** and **19** are potent, efficacious cardiotonic agents with approximately 10–20-fold greater potency than that of I and comparable or better potency than that of milrinone.

In further studies of 4-substitution, β-carbomethoxyethyl derivative **24** was prepared and had activity on the same order as that of the 4-propyl compound **14** (Table II). Encouraged by this initial result, we prepared carboxylic acid **25**, isopropyl (**32**) and ethyl (**33**) esters, amide **35**, methylamide **37**, and dimethylamide **38**, but all had significantly less interesting activity. Shortening the alkyl chain to carbomethoxymethyl (**36**) or to carbomethoxy (**39**) also reduced potency. Examination of substitution at C-1 with hydrogen (**31**), ethyl (**27**), propyl (**29**), or isopropyl (**30**) reduced potency as well. In contrast to the examples in Tables I and II, 6,7-methylenedioxy substitution (**28**) maintained good activity. Of these carboxylic acid derivatives, only **24** and **28** were as interesting as the 4-alkyl compounds described herein.

Two acyl derivatives of the 3-hydroxy function were prepared, namely, carbonate **41** and acetate **42** (Table IV). Comparison of **41** with **1** and **42** with **17** shows that activity and potency is maintained or somewhat enhanced by acylation of the hydroxyl group. It is not clear whether this activity is a result of **41** and **42** acting as prodrugs or from inherent activity of the acyl derivatives.

Data in Table II for the 4-alkyl compounds with the best iv potency (**15**, **16**, and **19**) as well as for the most interesting 4-(carboxyalkyl) derivative (**24**) shown in Table III indicate that these compounds also have cardiotonic activity when administered id albeit with lesser potency than that observed during iv dosing. This suggests that this isoquinolin-3-ol series may well have oral activity in further studies.

We have reported the relationship between cardiotonic activity and PDE-III enzyme inhibition for the series of compounds related to bemarinone.[3] To examine this relationship for isoquinolin-3-ols, a selected group of compounds with an interesting range of cardiotonic activity was studied. The log ED_{50} values for increasing contractile force (CF) and log IC_{50} values for enzyme inhibition are compared in Table V and plotted graphically. As may be seen from the graph in Figure 1, there appears to be a good linear relationship between the log ED_{50}–log IC_{50} pairs ($r = 0.87$). This result obtained from this data sample should be applicable to the entire series and suggests that similar structural requirements are in play for both activities and are similar to those discussed for bemarinone.[3] These include a flat aromatic ring nucleus, a limited "alkyl" pocket at C-1, a dipole region at C-3, and a hydrogen bonding region at C-7. As noted in our earlier SAR discussion, there are also differences of structural requirements between the two systems, the origins of which are unclear. While PDE-III enzyme inhibition predicted the best cardiotonics in the series, namely **15**, **16**, and **19**, these studies do not preclude the possibility of additional mechanisms of action.

A major characteristic of the entire group of compounds reported herein is their very short duration of action. The best compounds (**15**, **16**, **19**) all had excellent cardiotonic activity which rapidly returned to control values within 1 h of dosing. Whether the short duration of action is caused by rapid metabolism or clearance from the system is not known. Structural modifications of the isoquinolin-3-ol series in attempts to address these issues are the subjects of future reports from these laboratories.[15]

Experimental Section

Melting point determinations were done on either a Mel-Temp or Thomas-Hoover capillary melting point apparatus and are uncorrected. All compounds had spectra (IR, UV, ^{1}H NMR, and MS) consistent with their assigned structures and were homogeneous by thin-layer chromatography (TLC). Combustion analyses for C, H, and N were within ±0.4% of theory unless noted otherwise. Compounds in the tables were prepared according to the general procedures described below. Physical properties of the compounds are summarized in Tables I–IV.

Procedure A. (a) 6,7-Dimethoxy-1-methylisoquinolin-3-ol (1). To a mechanically stirred mixture of methyl 3,4-dimethoxyphenylacetate (4.2 g, 20 mmol) and acetic anhydride (10 mL, 106 mmol) with cooling in an ice bath was added 70% perchloric acid (2 mL) over a period of 7 min. The thick crystalline slurry was stirred at room temperature for 15 min and diluted with ether (80 mL). The yellow-brown solid was collected by filtration, washed with ether, and air-dried to give the benzopyrylium perchlorate salt, 5.4 g, 81%, mp 197–198 °C dec; IR (KBr) 1633 cm^{-1}; ^{1}H NMR (TFA) δ 4.72, 7.30, 7.13 (each s, 3 H, aromatic *H*), 4.35, 4.27, 4.17 (each s, 9 H, OCH_3), 3.18 (s, 3 H, CH_3). To a mechanically stirred slurry of this salt (5.4 g, 15.84 mmol) in water (1 mL) with cooling in an ice bath was added concentrated ammonium hydroxide (100 mL) dropwise over a period of 20 min. (**CAUTION**: The dry salt reacts with concentrated ammonium hydroxide explosively! Initial slurrying in water significantly reduces this hazard.) The mixture was stirred at room temperature for an additional 30 min. The yellow solid was collected by filtration, washed with water, and air-dried to give crude **1** (3.6 g, 100%), mp 270–275 °C. Recrystallization from ethanol gave the pure product, 2.7 g, 78%, mp 282–284 °C (lit.[10] mp 286 °C dec); IR (KBr) 1642, 1565, 1498 cm^{-1}; ^{1}H NMR (TFA) δ 7.45, 7.30, 7.25(each s, 3 H, aromatic *H*), 4.15 (s, 6 H, OCH_3), 3.08 (s, 3 H, CH_3); MS, *m/z* 219 (M^+); UV (EtOH) λ (ε (M^{-1} cm^{-1})) 313 (1700), 296 (2900), 286 (3700), 253 38300), 217 (15000) nm. Anal. ($C_{12}H_{13}NO_3$) C, H, N.

(b) 6,7-Dimethoxy-4-isopropyl-1-methylisoquinolin-3-ol (19). A solution of isopropyl (3,4-dimethoxyphenyl)isopropylacetate (4.55 g, 16.23 mmol) in acetic anhydride (7.73 mL, 81.15 mmol) was treated with boron trifluoride etherate (3.96 mL, 32.46 mmol), and the mixture was warmed to 50 °C overnight. The mixture was cooled in an ice bath and 70% perchloric acid (4 mL) was added dropwise. Ether was added dropwise until a crystalline solid formed, which was collected by filtration, washed with ether, and air-dried to give the benzopyrilium perchlorate salt, 3.45 g, 52.5%, mp 142–144 °C; IR (KBr) 1621, 1550, 1500 cm^{-1}. A stirred slurry of the salt (3.45 g, 8.52 mmol) in water (5 mL) was treated with concentrated ammonium hydroxide (8 mL) with ice-bath cooling over a period of 30 min. The yellow precipitate was collected by filtration, washed with water, and dried to give crude **19**, 2.07 g, 93%. The material was purified on a silica gel column with 3% ethanol in ethyl acetate as the eluent to give 844 mg of a solid which was recrystallized from methanol to give pure **19**, 470 mg, 31%, mp 223–225 °C; IR (KBr) 1631, 1485, 1440 cm^{-1}; ^{1}H NMR (TFA) δ 7.67 and 7.48 (each s, 2 H, aromatic *H*), 4.23 and 4.18 (each s, 6 H, OCH_3), 3.01 (s, 3 H, C H_3), 2.01 (m, 1 H, $CH(CH_3)_2$), 1.68 and 1.57 (each s, 6 H, $CH(CH_3)_2$); MS, *m/z* 261 (M^+). Anal. ($C_{15}H_{19}NO_3$) C, H, N.

Procedure B. 4-Chloro-6,7-dimethoxy-1-methylisoquinolin-3-ol (18). Compound **1** (0.877 g, 4 mmol) and *N*-chlorosuccinimide (0.588 g, 4.4 mmol) were dissolved with heating in CH_2Cl_2 (80 mL), and the mixture was heated for 16 h. The slurry was diluted with CH_2Cl_2 (50 mL) and cooled to room temperature, and the yellow solid was isolated by filtration. The product was triturated with $CHCl_3$ (250 mL) at reflux temperature, recollected by filtration, and washed with $CHCl_3$ to give **18**, 0.725 g, 71%, mp >300 °C; ^{1}H NMR (TFA) δ 7.57 and 7.53 (each s, 2 H, aromatic *H*), 4.27 and 4.20 (each s, 6 H, OCH_3), 3.13 (s, 3 H, CH_3); MS, *m/z* 253 (M^+). Anal. ($C_{12}H_{12}ClNO_3$) C, H, N, Cl.

Procedure C. 3-[(Methoxycarbonyl)oxy]-6,7-dimethoxy-1-methylisoquinolin-3-ol (41). Sodium hydride (0.17 g, 50%

(15) Kanojia, R. M.; Lever, O. W., Jr.; Press, J. B.; Williams, L.; Werblood, H. M.; Tobia, A. J.; Falotico, R.; Moore, J. B., Jr. *J. Med. Chem.*, submitted for publication.

suspension prewashed with hexanes, 7.14 mmol), and 1 (1.00 g, 4.76 mmol) were dissolved in dimethylformamide (50 mL) and the solution was cooled to −20 °C. Methyl chloroformate (0.50 g, 5.24 mmol) was added and the mixture was stirred for 30 min. The reaction mixture was quenched with water (50 mL) and extracted with methylene chloride (4 × 100 mL). The organic layer was washed with water (3 × 100 mL) and saturated brine (2 × 50 mL), dried over sodium sulfate, and evaporated to give crude 41, 0.95 g, 72%. Recrystallization from methanol gave the analytical material, 0.42 g, 44%, mp 147–149 °C; IR (KBr) 1760 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.18, 7.13, 6.98 (each s, 3 H, aromatic *H*), 4.00 and 3.98 (each s, 6 H, OCH_3), 2.85 (s, 3 H, CH_3); MS, *m/z* 277 (M^+). Anal. ($C_{14}H_{15}NO_5$) C, H, N.

Cardiotonic Activity.[13] Adult mongrel dogs were anesthetized with sodium pentobarbital (45 mg/kg, ip) and artificially respired. Mean arterial pressure (MAP) was recorded from a cannulated femoral artery, and drugs were infused into a cannulated femoral vein. The arterial pressure pulse was used to trigger a cardiotachometer for determination of heart rate (HR). Left ventricular pressure was measured with a Millar catheter and dP/dt_{max} was derived. A right thoracotomy was performed and myocardial contractile force (CF) was measured with a Walton Brodie strain gauge sutured to the right ventricle. The ventricular muscle was stretched to produce a baseline tension of 100 g. A catheter was inserted 2 cm distal to the pyloric valve via a flank incision for id drug administration. A standard dose of dopamine (10–15 μg/kg per min for 3 min) was administered to determine myocardial responsiveness to inotropic stimulation.

Test compounds were solubilized in a small volume of DMF and diluted to a final concentration of 10% in physiological saline. Alternatively, where possible, a soluble hydrochloride salt was prepared by addition of 0.1 N HCl diluted in physiological saline. Vehicles were tested in appropriate volumes and found to exert less than a 5% effect on contractile force. For iv studies, compounds were administered by infusion pump (one drug per animal) at rates of 0.58–2.2 mL/min in three to four stepwise increasing doses. Each dose was infused over 5 min immediately after the effect of the previous dose peaked. For id studies, compounds were injected into the duodenum through an indwelling catheter in a 10-mL bolus. MAP, HR, dP/dt_{max}, and CF responses were continuously monitored on a Beckman or Gould recorder and expressed as a percent change from predrug control values vs the cumulative dose of drug administered. For these studies, *n* of 1–4 test animals were used with variability ≤±10% based on historical base-line determinations in our laboratories.

Quantitation of the inotropic potency was obtained by calculation of the contractile force (CF) ED_{50}. This was defined as the dose of compound that produced a 50% increase above base line in myocardial contractile force. The value was obtained from three to four point dose–response curves by using either graphical estimation ($n < 3$) or linear regression analysis ($n \geq 3$).

Dog Heart Nucleotide Phosphodiesterase Fraction III (PDE-III). The enzyme preparation was obtained by using the method described by Thompson.[14] Briefly, a crude supernatant was chromatographed on a DEAE-cellulose column equilibrated with a sodium acetate buffer (pH 6.5) containing 30% ethylene glycol. The third fraction eluted from the column (at 800 mM sodium acetate) was the enzyme used for these studies. The enzyme was dialyzed and stored, until used, at −20 °C in 70 mM sodium acetate buffer (pH 6.5) containing 30% ethylene glycol. Potential inhibitors were tested at several concentrations (0.10 μM to 1 mM) in the presence of cyclic AMP (0.25 μM, containing 0.2 μCi [^3H]-cyclic AMP), enzyme, and 0.05 M Tris·HCl buffer at pH 7.4 containing 5 mM $MgCl_2$. The reaction was quenched by heating to 100 °C for 1 min. After cooling, 0.10 mL of a solution containing snake venom (1 mg/mL) was added and the reaction was allowed to proceed for 30 min. This enzymatic reaction converted all of the phosphodiesterase product to an uncharged compound (adenosine). Termination of this reaction was accomplished by the addition of 1.0 mL of 33% Dowex AGIX8 slurry to separate the product from unconverted substrate. An aliquot was removed from the resulting supernatant and [^3H]adenosine was quantitated by liquid scintillation spectrometry. Activity of the inhibitor is expressed as an IC_{50} value, the micromolar concentration of compound required to inhibit 50% of the PDE-III activity. Experiments were performed in duplicate on a single enzyme preparation with multiple trials using several standards as internal controls. The IC_{50} values are the result of at least two separate determinations at inhibitor concentrations from 0.01 to 1000 μM and are reported with a precision of ±15% (SEM) or better and are summarized in Table V.

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Registry No. 1, 16535-98-1; **2**, 114130-58-4; **3**, 114130-59-5; **4**, 114130-60-8; **5**, 114130-61-9; **6**, 114130-62-0; **7**, 114130-63-1; **8**, 114130-64-2; **9**, 114130-65-3; **10**, 114130-66-4; 11, 114130-67-5; **12**, 114130-68-6; **13**, 114130-69-7; 14, 114130-70-0; **15**, 114130-71-1; **16**, 114130-72-2; **17**, 114130-73-3; **18**, 114130-74-4; **19**, 114130-75-5; **20**, 114130-76-6; **21**, 114130-77-7; **22**, 114130-78-8; **23**, 114130-79-9; **24**, 114130-80-2; **25**, 114130-81-3; **26**, 114130-82-4; **27**, 114130-83-5; **28**, 114130-84-6; **29**, 114130-85-7; **30**, 114130-86-8; **31**, 114130-87-9; **32**, 114130-88-0; **33**, 114130-89-1; **34**, 114130-90-4; **35**, 114130-91-5; **36**, 114130-92-6; **37**, 114130-93-7; **38**, 114130-94-8; **39**, 114130-95-9; **40**, 114130-96-0; **41**, 114130-97-1; **42**, 114130-98-2; PDE-III, 9040-59-9; 3,4-$(OMe)_2C_6H_3CH_2CO_2Me$, 15964-79-1; 3,4,5-$(OMe)_3C_6H_2CH_2CO_2Me$, 2989-06-2; 3,5-$(OMe)_2C_6H_3CH_2CO_2Me$, 6512-32-9; *m*-$MeOC_6H_4CH_2CO_2Me$, 18927-05-4; 3-OMe, 4-$OtZC_6H_3CH_2CO_2Me$, 55761-07-4; 3-OMe, 4-$OBuC_6H_3CH_2CO_2Me$, 114130-99-3; 2,3-$(OMe)_2C_6H_3CH_2CO_2Me$, 27466-90-6; 3,4-$(OEt)_2C_6H_3CH_2CO_2Me$, 92157-09-0; *p*-$MeOC_6H_4CH_2CO_2Me$, 23786-14-3; 3,4-$(OMe)_2C_6H_3CH(Pr)CO_2Me$, 114131-00-9; 3,4-$(OMe)_2C_6H_3CH(Et)CO_2Me$, 114131-01-0; 3,4-$(OMe)_2C_6H_3CH(Me)CO_2Me$, 29207-02-1; 3,4-$(OMe)_2C_6H_3CH(Bu\text{-}i)CO_2Me$, 114131-02-1; 3,4-$(OMe)_2C_6H_3CH(C_5H_{11})CO_2Me$, 114131-03-2; 3-OMe, 4-$OEtC_6H_3CH(Et)CO_2Me$, 114131-04-3; 3,4-$(OMe)_2C_6H_3CH(CO_2Me)(CH_2)_2CO_2Me$, 114131-05-4; 3,4-$(OMe)_2C_6H_3XH(CO_2Me)(CH_2)_2CO_2H\cdot HCl$, 114131-06-5; 3,4,5-$(OMe)_3C_6H_2CH(CO_2Me)(CH_2)_2CO_2Me$, 114131-07-6; 3,4-$(OMe)_2C_6H_3CH(CO_2Me)(CH_2)_2CO_2Pr\text{-}i$, 114131-08-7; 3,4-$(OMe)_2C_6H_3CH(CO_2Me)(CH_2)_2CO_2Et$, 114131-09-8; 3,4-$(OMe)_2C_6H_3CH(CO_2Me)(CH_2)_2CONH_2$, 114131-10-1; 3,4-$(OMe_2C_6H_3CH(CO_2Me)CH_2CO_2Me$, 114131-11-2; 3,4-$(OMe)_2C_6H_3CH(CO_2Me)(CH_2)_2CONHMe$, 114131-12-3; 3,4-$(OMe)_2C_6H_3CH(CO_2Me)(CH_2)_2CONMe_2$, 114131-13-4; 3,4-$(OMe)_2C_6H_3CH(CO_2Me_2$, 100613-73-8; 3,4-$(OMe)_2C_6H_3CH(CO_2Me)CONHMe$, 114131-14-5; methyl 3,4-dimethoxyphenylacetate, 15964-79-1; benzopyrylium perchlorate salt, 71655-15-7; isopropyl (3,4-dimethoxyphenyl)isopropylacetate, 114131-15-6; 5-benzodioxoleacetic acid methyl ester, 326-59-0; milrinone, 78415-72-2; bemarinone, 92210-43-0; 5-[1,3-(dimethoxycarbonyl)propyl]benzodioxole, 114131-16-7.